Citation:

Farouque HM, Leung M, Hope SA, Baldi M, Schechter C, Cameron JD, Meredith IT. Acute and chronic effects of flavanol-rich cocoa on vascular function in subjects with coronary artery disease: a randomized double-blind placebo-controlled study. *Clin Sci (Lond)*. 2006 Jul;111(1):71-80.

PubMed ID: 16551272

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

The aim of this study was to determine the effects of flavanol-rich cocoa on invasive and non-invasive measurements of endothelial and vascular function over a 6-week period in patients with chronic ischaemic heart disease using a randomized double-blind placebo-controlled study design.

Inclusion Criteria:

Subjects had angiographically documented CAD (coronary artery disease; >50% stenosis in at least on epicardial coronary artery) and were clinically stable for at least 3 months before study enrollment with no modifications made to medication.

Exclusion Criteria:

Exclusion criteria included age <18 or >80 years, uncontrolled diabetes mellitus or significant non-cardiac medical illnesses.

Description of Study Protocol:

Recruitment: No specifics provided on how subjects were recruited.

Design: Randomized double-blind placebo-controlled trial

Blinding used: Study products were provided to subjects in unmarked silver wrappers. The study was double-blind.

Intervention:

Subjects received either a flavanol-rich chocolate bar and cocoa beverage daily (flavanol group: 444 mg of flavanols daily, ≈170 mg of epicatechin monomer daily) or matching isocaloric placebo daily [placebo (non-flavanol) group: 19.6 mg of flavanols daily; ≈4.7 mg of epicatechin monomer daily] for 6 weeks. The placebo chocolate bar and beverage had the same macronutrient, caffeine and theobromine content as the flavanol-rich cocoa products. To examine for acute effects, conduit vessel endothelial function was assessed before and 90 minutes after consumption of the first test meal (either a single flavanol-rich cocoa beverage or isocaloric placebo).

Statistical Analysis:

- Mann-Whitney *U* test was used to compare continuous variables between groups
- Pearson X^2 test was used to compare categorical variables

Data Collection Summary:

Timing of Measurements:

- All subjects underwent screening by history, physical examination, haematological and biochemical analyses and ECG. After screening, subjects received, in random order both a flavanol-rich chocolate bar and cocoa beverage daily or matching isocaloric placebo daily for 6 weeks. All study measures were performed at baseline and at 6 weeks.
- To examine for acute effects, conduit vessel endothelial function was assessed before and 90 minutes after the consumption of the first test meal. An interim assessment of conduit vessel endothelial function and SAC (systemic arterial compliance) was performed at 3 weeks. Subjects underwent vascular studies in the fasting state (with the exception of the cocoa beverage used in the acute 90 minute study) and at the same time in the morning to minimize the effect of diurnal fluctuations in vascular reactivity. Clinical review was performed on a weekly basis to ensure compliance with the treatment.
- Brachial artery FMD (flow-mediated dilation) reactivity was assessed at baseline, 90 minutes after the first test meal and at 3 and 6 weeks. Strain-gauge VOP (venous occlusion plethysmography) was performed. FBF (forearm blood flow) was measured to assess endothelium-dependent and -independent vasodilation respectively. SAC was measured non-invasively; subjects were studied in the supine position at baseline, 90 minutes after the first meal and at 3 weeks and 6 weeks after study commencement.
- Circulating biomarkers of endothelial function, including soluble ICAM-1 (intercellular cell-adhesion molecule-1), VCAM-1 (vascular cell adhesion molecule-1), P-selectin and E-selectin, were assayed in duplicate using commercially available ELISA kits. High sensitivity CRP (C-reactive protein), total cholesterol, serum triacylglycerols, LDL, HDL, lipoprotein (a), serum glucose and plasma insulin levels were checked at 6 weeks. Blood samples for epicatechin determinations were taken at baseline and at 90 minutes after the first cocoa beverage.

Dependent Variables

- Invasive and non-invasive measurements of endothelial and vascular function, including total cholesterol, LDL- and HDL-cholesterol, triacylglycerols, CRP, lipoprotein (a), VCAM-1, ICAM-1, E-selectin and P-selectin

Independent Variables

- Flavanol-rich chocolate bar and cocoa beverage daily

- Matching isocaloric placebos daily

Control Variables

Description of Actual Data Sample:

Initial N: 40 subjects

Attrition (final N): 38 subjects

Age: Placebo group 61±8 years, Flavanol group 61±9 years

Ethnicity: Not noted

Other relevant demographics: Not noted

Anthropometrics:

Baseline characteristics of the two groups

Variable	Placebo group	Flavanol group	*P* value
n	20	20	
Obesity (n)	3 (51%)	4 (20%)	0.622
Diabetes (n)	2 (10%)	1 (5%)	0.548
Hypertension (n)	12 (60%)	10 (50%)	0.525
Current smoker (n)	2 (10%)	0 (0%)	0.147
Hypercholesterolaemia (n)	20 (100%)	19 (95%)	0.311
Family history CHD (n)	7 (35%)	7 (35%)	1.000
CHD risk factors (n)			
None	0 (0%)	1 (5%)	---
One	4 (20%)	6 (30%)	0.617
Two	9 (45%)	8 (40%)	---
Three	7 (35%)	5 (25%)	---
Angina (n)	4 (20%)	3 (15%)	0.677
Previous PCI (n)	13 (65%)	16 (80%)	0.288
Previous CABG (n)	8 (40%)	6 (30%)	0.507

Medication			
Aspirin Treatment (n)	19 (95%)	19 (95%)	1.000
Clopidogrel (n)	1 (5%)	2 (10%)	0.548
ACE-inhibitor (n)	11 (55%)	7 (35%)	0.204
β-Blocker (n)	10 (50%)	8 (40%)	0.525
Nitrates (n)	2 (10%)	3 (15%)	0.633
Calcium channel blocker (n)	1 (5%)	4 (20%)	0.151
Statin (n)	19 (95%)	16 (80%	0.151
Mean height (m)	1.73±0.08	1.70±0.09	0.376
Mean weight (kg)	84±11	79±16	0.384
BMI (kg/m^2)	27.9±2.9	27.1±3.9	0.555
Diastolic BP (mmHg)	82±10	77±11	0.126
Systolic BP (mmHg)	136±15	131±19	0.632
Mean BP (mmHg)	100±11	95±12	0.186

The group receiving flavanol-rich cocoa had higher baseline total cholesterol (P = 0.013) and LDL-cholesterol (P = 0.005) compared with the placebo group.

Location: Australia

Summary of Results:

Key Findings

- There were no differences in baseline demographic and morphometric characteristics
- The duration of treatment in each group was similar (42.3±1.7 days in the flavanol group compared with 42.7±1.0 days in the placebo group; *P*=0.42)
- One subject (5%) dropped out after the acute FMD study
- There was no change in weight during the study (0.40±0.35 kg in the flavanol group compared with 0.68±0.49 kg in the placebo group; *P*=0.487)
- Plasma epicatechin concentration increased acutely in all subjects receiving the flavanol-rich cocoa beverage but in only five subjects receiving the placebo beverage (mean increase of 153.7 nmol/l in the flavanol group compared with 2.9 nmol/l in the placebo group; *P*<0.0001)
- There was no difference in the change in brachial artery diameter and blood flow induced by post-ischaemic hyperaemia and GTN at baseline
- Similar to the FMD response, the evolution over time of the endothelium-independent (SNP) FBF dose-response curves showed no significant difference average over the doses of SNP [change in FPF at 6 weeks in the flavanol group compared with the placebo group = -0.53 (95% CI, -1.28 to +0.22), where CI is confidence interval]. However, the

enothelium-dependent (ACh) FBF dose-response curves differed slightly between the flavanol-rich cocoa and placebo groups averaged over doses of ACh [change in FBF at 6 weeks in the flavanol group compared with the placebo group = -1.61 (95% CI, -2.78 to -0.4268)]

- There was a significant difference at the highest concentrations of ACh between baseline and 6 weeks in the placebo group [change in FBF at 6 weeks = 1.44 (95% CI, +0.13 to +2.75) and 1.92 (95% CI, +0.62 to +3.23) at doses of 10 and 30 µg/min ACh respectively]
- At 6 weeks, forearm hyperaemic responses following a brief period of exercise or ischaemia were not altered by the ingestion of flavanol-rich cocoa compared with the baseline state
- There was no difference in systolic, diastolic or mean arterial pressure, heart rate or SAC in the groups at baseline
- Acute ingestion of flavanol-rich cocoa did not alter haemodynamics or SAC nor was SAC affected by long-term ingestion of either dietary supplement [SAC in the flavanol group - SAC in the placebo group = -0.010 (95% CI, -0.029 to +0.010), 0.017 (95% CI, -0.004 to +0.038), 0.006 (95% CI, -0.015 to +0.028) and -0.003 (95% CI, -0.025 to +0.018) at baseline, 90 minutes, 3 weeks and 6 weeks respectively
- CRP, ICAM-1, E-selectin and P-selectin did not differ between the two groups at baseline or following 6 weeks of daily chocolate consumption
- VCAM -1 levels were lower in the placebo (non-flavanol) group at baseline but did not change appreciably with 6 weeks of treatment.

Relative changes in brachial artery diameter and blood flow to FMD and GTN in the flavanol group compared with the placebo (non-flavanol) group at baseline, after 90 minutes (acute) and after 3 and 6 weeks

	Brachial artery diameter	Blood flow
Baseline Post-ischaemic hyperaemia GTN	-3.07(-6.95 to +0.82) -3.917(-0.47 to +8.29)	16.33(-914.2 to +946.9) -58.14(-12.00 to +128.3)
At 90 minutes Post-ischaemic hyperaemia GTN	4.85(-0.40 to 10.10) -0.20(-5.60 to +5.20)	-655.3 (-1770.3 to +459.6) 58.76 (-149.8 to +32.2)

At 3 weeks Post-ischaemic hyperaemia GTN	1.53(-3.79 to +6.85) -2.63 (-8.12 to 2.85)	-534.53 (-598.3 to +1667.2) -30.18 (-122.6 to 62.20)
At 6 weeks Post-ischaemic hyperaemia GTN	3.57 (-1.75 to 8.89) -1.37 (-7.85 to +4.12)	-432.43 (700.3 to +1565.2) -40.34 (132.7 to +52.04)

Author Conclusion:

- The major finding was that flavanol-rich cocoa taken daily over a 6 -week period was safe, but did not improve endothelial function or SAC in patients with multiple cardiovascular risk factors and advanced coronary arteriosclerosis. Furthermore, flavanol-rich cocoa did not alter peripheral conduit vessel endothelial function 90 minutes after ingestion of a flavanol-rich cocoa beverage
- Flavanol-rich cocoa or chocolate supplementation did not change conduit vessel arterial function in the acute or chronic setting
- Examination of forearm resistance vessel function demonstrated that both endothelium-dependent and -independent responses were similar at baseline and were unchanged by 6 weeks of flavanol-rich cocoa consumption
- No significant treatment effect on systolic or diastolic BP, mean arterial pressure or heart rate was observed
- The use of concomitant vasoactive medication for the treatment of hypertension and ischaemic hear disease in the present study population may not have offset any potential antihypertensive effect of flavanol-rich cocoa
- No reduction in soluble ICAM-1, VCAM-1, E-selectin or P-selectin levels were noted despite 6 weeks of flavanol-rich cocoa supplementation
- Circulating oxidizing LDL, a measure of oxidant stress was also unchanged
- The lack of effect of flavanol-rich cocoa in improving any variables suggest that the findings are perhaps less likely to be a result of type 2 statistical error
- Compliance to the cocoa products was excellent and the total flavanol content within the cocoa products used in the study (444 mg/day) was comparable with that used in other studies (176-821 mg/day) in which improvements of peripheral vascular function were noted
- The findings of the present study do not support an incremental benefit of flavanol-rich cocoa on vascular function in subjects with CAD receiving typical therapies for this condition and its associated risk factors.

Reviewer Comments:

- *It is possible that the age of subjects and their burden of cardiovascular risk factors and vascular disease may have been too great for flavanol-rich cocoa to exert a positive effect over the time frame of the study*
- *Intervention only lasted 6 weeks*
- *Significant differences between groups at baseline in terms of total and LDL cholesterol*
- *Potential differences in baseline dietary flavanoid intake or the use of background medication in the subjects with chronic ischaemic heart disease, which are known to improve vascular function, may also have potentially masked any benefit of flavanol-rich cocoa.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes

2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	???
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	???
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A

5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	Yes
6.6.	Were extra or unplanned treatments described?	Yes
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes

	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		???
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	???